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Ns- 1 Ag Detection – An Early Marker for Dengue Viral Infection

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ABSTRACT

Background: Dengue fever is one of the most common mosquito borne arboviral illness in India. It has become a public health problem in tropical countries nowadays. Early and accurate detection of Dengue virus infection from acute fever patients is very important in patient management and for control measures in outbreaks. **AIMS & OBJECTIVE:** To evaluate the efficacy of NS-1 Ag detection in the diagnosis of acute dengue infection and to compare it with IgM capture ELISA and PCR. **MATERIALS & METHODS:** A total of 82 serum samples were received from patients with fever of 4-7 days, from Government Rajaji Hospital, Madurai, during the April -May outbreak in 2012. NS 1 Ag detection was done using [Panbio ELISA Kit]. IgM antibody detection by [NIV, Pune, ELISA Kit].All the positive samples were then confirmed by Polymerase Chain Reaction. RESULTS: Out of the 82 samples tested, 22 samples were positive by IgM Capture Elisa and 60 were negative. When tested by NS -1 Antigen Elisa kit, among the 82, 31 showed positivity and 51 were negative.PCR was performed to confirm positivity, 22 samples tested by IgM Capture Elisa and all the 31 samples tested by NS -1 Antigen Elisa were positive. **Conclusion**: Thus the present study shows that NS 1 Ag detection helped in early diagnosis so as to initiate prompt treatment and prevent complications. NS 1 Ag detection serves as a better approach to diagnosis of Dengue virus Infection.

KEYWORDS

INTRODUCTION:

Dengue viral infection is one of the common arboviral infections in India. It is a mosquito-

borne disease caused by flavivirus of the family Flaviviridae. It is an enveloped, single-stranded, positive-sense RNA virus .Dengue virus (DEN) has four different serotypes. They are(DEN1, DEN2, DEN3, and DEN4). Recently it has been considered as a re-emerging infection because of the dramatic increase in recent decades. There is an estimated annual occurrence of 100 million new cases in tropical and subtropical regions of the world .[1,2,3] . Infection with any of the four serotypes of dengue virus causes a spectrum of clinical features ranging from asymptomatic infections, undifferentiated fever, and classical Dengue Fever (DF) to life-threatening manifestations such as Dengue Hemorrhagic Fever (DHF) and dengue shock syndrome (DSS), which are often due to reinfection by heterologous serotypes.[4] Infection with one serotype provides lifelong immunity against homologous reinfection. Protection against subsequent infection by the other three serotypes is only partial and transient. Therefore, people who live in epidemic areas may be susceptible to four infections in their lifetime. Seroepidemiological studies have shown that subsequent heterologous infections may increase the risk of development of more-severe manifestations.[5] At present, however, there is no protective vaccine or specific treatment available for Dengue viral infections. Early definitive diagnosis of dengue virus infection may help in the timely management of dengue virus infection Thus, early clinical management can reduce the morbidity and mortality of DHF or DSS.

In order to differentiate Dengue fever from other febrile illnesses and hemorrhagic fever,   a definite microbiological diagnosis of Dengue Viral infections is essential. A rapid and accurate dengue diagnosis in the acute phase of illness is important for early enhancement of epidemiological control measures in areas with low endemicity. Furthermore, for epidemiological and pathological investigations, it is important to determine the correlations of different DEN serotypes with disease severity.[6] Currently, laboratory diagnosis of DengueV-

iral infections is based on virus isolation, serology, and RNA detection. Virus isolation is the "gold standard" for diagnosis and serotyping of Dengue viral infections, but this method is time consuming and requires a sophisticated laboratory. Viral nucleic acid detection typically provides more sensitive and rapid diagnosis than the traditional virus isolation method. However, molecular diagnoses, such as reverse transcriptase (RT) PCR, require experienced technicians and specialized laboratory equipment.Moreover false-positive results due to amplifications that carry over contamination are not uncommon. Although the detection of antibodies with whole virus antigen-based enzyme-linked immunosorbent assay (ELISA)is most commonly used, the limitations of this assay are crossreactivity with all serotypes of Dengue Virus as well as other members of the Flavivirus family, especially in cases of secondary Dengue Viral infection .[7,8]Therefore, early diagnosis and determination of the serotype still remains a problem, as it mainly depends on RT-PCR or virus isolation methods.

The detection of viral antigens has been focused on nonstructural protein 1 (NS1) of Dengue virus.[9,10,11] This protein has been identified as a highly conserved glycoprotein expressed in either membrane-associated or secreted forms. It possesses not only group-specific but also type-specific determinants and has been recognized as an important antigen in Dengue Viral infections[8,12,13] Therefore, the NS1 Antigen detections can be used as a serotyping marker and an early diagnostic tool. A high circulating level of NS1 was demonstrated in the acute phase of dengue by antigen capture ELISA.[9,11]

MATERIALS AND METHODS:

A total of 82 serum samples were received from patients with fever of 4-7 days, from Government Rajaji Hospital, Madurai, during the April -May outbreak in 2012. NS 1 Ag detection was done using [Panbio ELISA Kit] . IgM antibody detection by [NIV, Pune, ELISA Kit]. All the positive samples were then confirmed by Polymerase Chain Reaction.

RESULTS

Out of the 82 samples tested,22 (26.8%) samples were pos-

itive by IgM Capture Elisa and 60 (73.2%) were negative. When tested by NS -1 Antigen Elisa kit, among the 82, 31 (37.8%) showed positivity and 51(62.2%) were negative.PCR was performed to confirm positivity of the 22 samples tested by IgM Capture Elisa and 31 samples tested by NS -1 Antigen Elisa . More positivity was detected by NS-1 Antigen ELISA in the earlier phase than IgM Capture Elisa .NS-1 Antigen assay holds good in early diagnosis.

DISCUSSION
A valuable tool in the early diagnosis of Dengue fever is NS-1 Antigen detection.NS 1 Ag is present from the day one of fever both in primary and secondary infections. The dengue-specific antibodies begin to appear only around fifth day of fever in primary infection.[14.] In most of secondary infections, both the IgM and IgG type antibodies cannot be recorded before third day.[15.] Antibody detection is an indirect method of diagnosis and, therefore, is prone to false positive as well as false negative results.[14.] So in this window period only NS1Ag can be detected. It is important to note that NS1 is shown to be a highly specific viral marker making it extremely reliable parameter for the diagnosis of Dengue infections from day 1 of the fever[16.]. When the precise day of fever at the time of conducting the test could not be obtained , NS1 only was positive in 30% cases. Given an opportunity to test every case of fever on day 1, more number of cases can been picked up by NS1 Antigen detection. It is shown that the titres of NS1 represent the viral load and the viral load is directly proportional to complications.[17.]

In this study,Out of the 82 samples tested,22 samples were positive by IgM Capture Elisa and 60 were negative .Simultaneous testing for NS -1 Antigen by Elisa , among the 82, showed 31 positivity and 51 were negative .Nine patients with dengue infection would have been missed , if NS -1Ag detection was not carried out on then. Polymerase Chain Reaction (PCR) can   be used for definite diagnosis of dengue fever in the early days of fever. But definitely not cost effective. ELISA has higher sensitivity than ICT-based tests.[18.]From this study , we conclude that NS -1Ag detection will be very useful for in outbreaks for early diagnosis and to prevent complications. Recently, commercially available kits for the detection of dengue virus NS1 antigen have been developed, and studies have shown that dengue virus NS1 antigen could be useful for the detection of early stages of dengue virus infections.[19,20,21,22,23.]

RESULTS

RESULTS	NS 1 Ag (%) n = 82	IgMCapture ELISA(%) n = 82	PCR(%) n = 82
POSITIVES	31(37.8)	22(26.8)	31(37.8)
NEGATIVES	51(62.2)	60(73.2)	51(62.2)

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